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VOLATILE CONSTITUENTS AND CHARACTER IMPACT COMPOUNDS OF SELECTED FLORIDA'S TROPICAL FRUIT

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Abstract. **The aroma compounds of Florida grown guava, mango and carambola were isolated by headspace SPME and by solvent extraction methods coupled with gas chromatography-mass spectroscopy (GC/MS) and GC-Olfactometry (GC-O). The volatile components were analyzed using both headspace and solvent extraction techniques in order to insure that the results of GC-O were representative of the fruit aroma. The combination of the two techniques resulted in 53, 48 and 46 aroma active compounds detected in carambola, guava and ripe mango, respectively. There was no single aroma character impact compound that contributed to the aroma of these fruit. The major classes of aroma active volatiles that contributed to the fruity aroma were the esters followed by aldehydes, alcohols, and ketones, which contribute green and sweet notes to fruit aroma. In addition, sulfur and norisoprenoid compounds contributed to the guava and carambola aromas, respectively.**

The Florida grown tropical fruit, carambola (*Averrhoa carambola*), guava (*Psidium guajava*) and mango (*Mangifera indica* cv. Keitt) have become popular due to their exotic flavor and nutritive benefits. They can be consumed fresh or processed into various products. There is published information identifying volatile compounds found in these fruits, but which ones contribute to flavor remains unclear.

Over 200 volatile components of carambola have been identified (MacLeod and Ames, 1990) with esters and lactones being significant components of carambola aroma along with several compounds which are derived from carotenoid precursors including four isomeric megastigma-4,6,8-trienes, and megastigma-5,8(E) and (2)-dien-4-one. Methyl anthranilate, which has a distinct grape-like aroma was identified in the 'Golden Star' variety, which possesses a grape-like aroma (Wilson et al., 1985). The warm fruity character of carambola fruit was attributed to ethyl acetate and ethyl butanoate (Wilson et al., 1985).

Over 154 different volatile compounds have been found in guava fruits (Idstein and Schreier, 1985). They have been identified as carbonyls, esters, alcohols, hydrocarbons, acids, sulfur-containing compounds and other miscellaneous volatiles. Askar et al. (1986) identified over 80 volatiles in guava and determined mycrene, *cis-* and *trans*-ocimene and β-caryophyllene to be the most important volatile compounds to guava aroma. The characteristic guava aroma is reported to be mainly the result of cinamyl derivatives, β-caryophyllene and C_6-derivatives of aldehyde (Taylor, 1993; Wilson and Shaw, 1978). Macleod and de Troconis (1982a) also reported that β-caryophyllene, 2-methylpropyl acetate, myrcene, hexyl acetate, benzaldehyde, ethyl decanoate, and α-selinene had guava-like odor. Stevens et al. (1970) reported methyl benzoate, methyl cinnamate, *p*-phenylethyl acetate, cinnamyl acetate, hexanone and β-ionone to be important.

Mango varieties differ in the amount and type of flavor compounds present, often depending on their origin. Generally, "Old World" (Asian) mangoes are reported to have more oxygenated aroma volatile compounds such as esters, furanones and lactones, giving some varieties pineapple or peach-like aromas, while "New World" mangoes that are hybrids of Old World stock have higher levels of certain hydrocarbons such as 3-carene (MacLeod and de Troconis, 1982b; Narain et al., 1988; Wilson et al., 1986), which are reportedly important for flavor (Malundo et al., 1996, 1997). It has been reported that monoterpenes and sesquiterpenes, the main compounds of "New World" mango aromas, represent 70-90% of total volatiles in some mango cultivars (Winterhalter, 1991).

Quantitatively, the major volatile component may or may not be important contributors to the aroma of a fruit, while minor components may have high odor activity. Many volatile components are not aroma active at all. Gas chromatography-olfactometry (GC-O), using the human nose as detector, is employed to determine whether a particular volatile, eluting from the GC, has aroma activity. Due to the possible variation in volatile compounds, caused by sample preparation method, headspace Solid Phase Microextraction (SPME) and standard solvent extraction methods were both used to assure that the results of GC-O were representative of the fruit aroma. The goal of the present study was to identify aroma-active compounds in guava, carambola and mango using both headspace (SPME) and solvent extraction of flavor volatiles coupled with GC/MS and GC-O.

Materials and Methods

Fruit samples. Carambola (*Averrhoa carambola*), red guava (*Psidium guajava*) and ripe mango (*Mangifera indica* cv. Keitt) were obtained from growers in South Florida. The edible part of each of 10 fruits for each fruit type was cut and flash frozen with liquid nitrogen and stored at -20°C for later analysis.

Headspace sampling. The frozen fruit was thawed, added to 30% NaCl (w/w), and homogenized in a Warring blender. A

Compounds	Aroma descriptor	RI	SPME	PE
Diacetyl[z]	Buttery	605	x	
Ethyl isobutyrate[z]	Fruity	708	x	
Methylbutanoate[z]	Fruity acidic	727	x	
Hexanal + Ethyl butanoate[z]	Green fruity	802	x	x
Ethyl 2-methylbutanoate[z]	Fruity sweet green	850	x	
2-(E)-Hexenal[z]	Fruity	854	x	x
2,4-Hexadienal[y]	Green fruity	905		x
1-Octen-3-one[z]	Mushroom	980	x	x
β-Myrcene[z]	Green herbal piney	984	x	x
Butyl butanoate[y]	Fruity minty	993	x	
Ethyl hexanoate[z]	Green sweet fruity	1000	x	x
α-Terpinene[y]	Green citrusy	1021		x
β-(E)-Ocimene[y]	Sweet herbaceous warm	1056	x	x
Terpinolene[y]	Fruity floral lemonate	1084	x	
Methyl benzoate[z]	Musty minty floral sweet	1096	x	x
Nonanal[z]	Minty floral	1103	x	x
3-Mercapto hexanol[y]	Sulfury tropical warm	1127		x
Nerol oxide[y]	Sweet fruity	1147		x
2,6 (E,Z) Nonadienal[y]	Sweet cucumber green	1155		x
2 (E) Nonenal[y]	Metallic green herbal	1161		x
1-Nonanol[y]	Green metallic	1171		x
Ethyl benzoate[z]	Tropical sulfury floral	1173	x	
2-Isobutyl-3-Methoxypyrazine[y]	Dried-chili bell pepper	1181		x
4-Methyl acetophenone[y]	Fresh sweet	1189		x
Ethyl octanoate[z]	Sweet floral	1192		x
Carvone[z]	Sweet minty spice	1244	x	
Bornyl acetate[y]	Sweet green	1282		x
δ-octalactone[z]	Sweet fruity	1287		x
Carvacrol[y]	Clove warm sweet spice	1293		x
Edulan1[y]	Metallic green sweet herbal minty floral	1315	x	
Piperonal[y]	Sweet floral rose nutty	1333		x
Ethyl dihydrocinnamate[y]	Sweet green floral honey	1350	x	
Megastigma-4,6(E), 8(E)-triene[z]	Floral sweet honey	1360	x	x
Unknown	Green sweet	1371	x	
2-methyl-2-ethyl-3 hydroxyhexyl propanoate[z]	Green sweet fruity honey	1378	x	x
β-Damascenone[z]	Floral sweet honey	1383	x	x
β-Damascone[y]	Honey	1409	x	x
Ethyl anthranilate[y]	Honey sweet floral	1413		x
β-Ionol[z]	Honey	1428	x	x
α-Ionone[y]	Honey	1437	x	x
Benzyl pentanoate[y]	Honey	1446	x	
Ethyl cinnamate[z]	Sweet spice green minty	1474	x	x
2-Phenylpropyl butyrate[y]	Sweet tropical	1482	x	
β-Ionone[z]	Sweet floral raspberry	1486	x	x
δ-Decalactone[y]	Sweet fruity spice coconut	1505		x
α-Farnesene[y]	Green sweet fruity	1507	x	
Geranyl isobutyrate[y]	Green sweet honey coconut	1518	x	x
2,5-bis (2-methylpropyl)-thiophene[z]	Sweet green fruity	1533	x	
Caryophyllene alcohol[y]	Sweet tropical green	1564	x	
Unknown	Green warm	1614		x
Unknown	Rancid burning	1668		x
Unknown	Fatty rancid	1682		x
Farnesol[y]	Sweet warm candy	1740		x

[z]Identified by linear retention index (RI) on HP-5 column, aroma description as compared with standard or literature values and mass spectrum.
[y]Tentatively identified by linear retention index (RI) on HP-5 column, aroma description as compared with standard or literature values.

5 g aliquot of fruit homogenate was added to a 20 mL glass vial, which was capped with a Teflon-coated septa cap. The vial was placed in a water bath at 40°C. After 15 min, a SPME fiber (50/30 µm DVB/Carboxen/PDMS on a 2-cm StableFlex fiber, (Supelco, Bellefonte, Pa.) was inserted into the headspace of the sample vial and exposed for 45 min. Subsequently, the fiber was thermally desorbed in the GC injector port for 5 min at 250°C.

Solvent extraction. The fruit homogenate (100 g) was extracted with 100 mL of a mixture of pentane: ether (1:1 v/v) in explosion proof Waring blender for 1 min. The mixture was centrifuged and the solvent layer collected. The residue

Table 2. Identification, aroma descriptors, retention index (RI) of aroma-active compounds in guava by GC-MS and GC-O using two sampling techniques, headspace SPME and solvent extraction (pentane-ether, PE).

Compounds*	Aroma descriptor	RI	SPME	PE
Acetaldehyde[z]	Solvent fruity	447	x	
Methanethiol[y]	Sulfury	685	x	
Ethyl isobutyrate[z]	Fruity	708	x	
Hexanal + Ethyl butanoate[z]	Green fruity	798	x	x
Butyl acetate[y]	Fruity	812	x	x
Butanoic acid[z]	Cat urine, sulfur	831	x	x
Butyric acid[y]	Cheesy	848		x
3-(Z)-Hexenol[z]	Green fruity	864	x	x
2-Methyl-3-furanthiol[y]	Nutty roasted	869	x	
Methylbutyric acid[y]	Sour sweaty	874	x	x
2,4-Hexadienal[y]	Green fruity	902	x	x
1-Octen-3-one[y]	Mushroom	977	x	x
β-Myrcene[z]	Green herbal piney	985	x	x
Butyl butanoate[y]	Sweet fruity	998	x	
Ethyl hexanoate[z]	Green fruity	1002	x	x
α-Terpinene[y]	Green citrusy	1026	x	x
Mercaptomethylbutyl formate[y]	Cut urine sweet	1029	x	
4-Mercapto-4-methylpentan-2-ol[y]	Grapefruit	1039	x	x
Furaneol[y]	Sweet caramel fruity	1060	x	x
1-Octanol[y]	Meaty oily	1065	x	x
Dimethylmethoxyfuranone[z]	Sweet caramel	1066		x
Guaiacol[y]	Roasted meat burnt	1089	x	x
Ethyl heptanoate[y]	Sweet fruity floral caramel furan	1093	x	
Methyl benzoate[z]	Minty floral caramel	1096	x	
Linalool[z]	Strong sweet floral	1101	x	x
3-Mercapto hexanol[y]	Grapefruit sulfury tropical warm	1131	x	x
3-Terpinen-1-ol[y]	Grapefruit	1138	x	x
Nerol oxide[y]	Fruity sweet	1146	x	x
2,6 (E,Z) Nonadienal[y]	Green fresh geranium	1153	x	x
Unknown	Sweet floral	1157	x	
2-(E) Nonenal[y]	Metallic green	1161	x	x
Ethyl octanoate[z]	Sweet fresh floral	1194		x
Carvone[z]	Sweet minty spice	1243	x	
Unknown	Sulfury warm tropical musty cat urine	1248	x	x
Unknown	Moldy	1253		x
Unknown	Moldy sweaty cat urine	1262		x
(E)-Cinnamaldehyde[z]	Cinnamon spicy	1277	x	x
δ-octalactone[y]	Sweet fruity	1290	x	
Edulan1[z]	Minty green sweet floral	1314	x	
Piperonal[y]	Floral nutty spice	1329		x
Ethyl dihydrocinnamate[z]	Sweet geramium floral	1348	x	x
Eugenol2	Sweet floral spicy clove cinnamon	1366	x	x
Unknown	Honey floral sweet warm	1374		x
β-Damascenone[z]	Honey floral sweet	1381	x	x
Ethyl anthranilate[y]	Honey floral sweet	1413	x	x
β-Caryophyllene[z]	Fruity warm floral	1434		x
Ethyl cinnamate[z]	Sweet floral spice	1473	x	x
β-Ionone[z]	Raspberry	1486	x	x

[z]Identified by linear retention index (RI) on HP-5 column, aroma description as compared with standard or literature values and mass spectrum.
[y]Tentatively identified by linear retention index (RI) on HP-5 column, aroma description as compared with standard or literature values.

was re-extracted, the solvent layers were combined and dried with DryDisk™ and subsequently concentrated to 0.1 mL using a Kuderna-Danish evaporative concentrator and followed by a flow of nitrogen gas. The extract 0.2 μL was introduced to the GC-MS.

GC-MS analysis. The separation of the volatile compounds was accomplished using an Agilent 6890 GC equipped with a 30 m × 0.25 mm × 0.25 μm HP-5 column, and coupled with a 5973N MS detector (Agilent, Palo Alto, Calif.). The column oven was programmed to hold at 50°C for 5 min, then to increase at 4°C/min to 250°C and hold for 15 min. Helium was used as carrier gas at flow rate of 2 mL·min⁻¹. Injector and ionizing source were kept at 250°C and 280°C, respectively. The split ratio was 1:1 with a 0.2 μL sample injection. The splitless mode was used when samples were introduced by SPME. Data were collected using the ChemStation G1701 AA data system (Hewlett-Packard, Palo Alto, Calif.).

GC-Olfactometry analysis. The GC condition was the same as mentioned above. The HP-5 column was connected to a sniff port (ODP-2, Gerstel, Inc., Baltimore, Md.). The split ratio was ⅓ to the MSD and ⅔ to the sniff port. Humidified air was run through the sniff port at a flow rate of 100 mL·min⁻¹. Two

Table 3. Identification, aroma descriptors, retention index (RI) of aroma-active compounds in mango by GC-MS and GC-O using two sampling techniques, headspace SPME and solvent extraction (pentane-ether, PE).

Compounds	Aroma descriptor	RI	SPME	PE
Ethanol[z]	Solvent-like	516	x	
Diacetyl[y]	Buttery	608	x	
Hexanal + Ethyl butanoate[z]	Green fruity	802	x	
Butyl acetate[y]	Fruity	815		x
Butyric acid[y]	Cheesy	841		x
Ethyl-2-methylbutanoate[z]	Fruity sweet	850	x	x
1-Hexanol[y]	Green musty nutty	870	x	
2-Methyl-3-furanthiol[y]	Nutty medicinal cooked oily	875		x
Methylbutyric acid[y]	Cheesy	893		x
Heptanal[z]	Nutty green	899	x	
2,4-Hexadienal[y]	Fruity green	907		x
Ethyl-3-hydroxy butanoate[y]	Green floral	943	x	
Benzaldehyde[y]	Sweet fruity	968		x
1-Octen-3-one[z]	Mushroom	978	x	x
β-Myrcene[z]	Green	988	x	x
Octanal[y]	Citrusy	1007		x
4-Mercapto-4-methyl penten-2-ol[y]	Green grapefruit	1040	x	
Phenylacetaldehyde[y]	Green pungent	1044		x
Homofuraneol[z]	Very sweet	1086	x	
Guaiacol[y]	Smoky	1090		x
Ethyl heptanoate[y]	Furan fruity sweet	1091	x	
Methyl benzoate[y]	Floral sweet	1097	x	x
Nonanal[z]	Sweet fruity	1101	x	x
Unknown	Sweet	1110	x	
Ethyl-3-hydroxyhexanoate[y]	Floral tabacco	1136		x
2 (Z) Nonenal[y]	Metallic	1147	x	
Nerol oxide[y]	Mild fruity	1149		x
2,6 (E,Z) Nonadienal[y]	Green geranium fresh sweet	1151	x	x
2 (E)-Nonenal[y]	Green metallic	1158	x	x
1-Nonanol[y]	Fresh metallic green	1168	x	x
α-Terpineol[y]	Sweet fruity	1185	x	x
Ethyl octanoate[y]	Sweet fruity	1204	x	
Unknown	Floral	1265	x	
1-Decanol[y]	Sweet metallic herbal	1279	x	
Unknown	Fatty sweet	1283	x	
δ-octalactone[y]	Fruity	1287		x
Undecanal[y]	Sweet	1303		x
Edulan1[y]	Green fresh minty	1313	x	
Methyl decanoate[y]	Green fruity	1328		x
t-Carvyl acetate[y]	Sweet green tea fruity	1335	x	x
α-Terpinyl acetate[y]	Sweet	1345		x
Neryl acetate[y]	Sweet caramel fruity floral	1357	x	x
Butyl butanoate[y]	Floral	1376		x
β-Damascenone[z]	Strong honey fruity floral tobaco	1386	x	x
δ-Decalactone[y]	Fruity sweet	1474	x	
β-Ionone[z]	Raspberry	1486	x	x

[z]Identified by linear retention index (RI) on HP-5 column, aroma description as compared with standard or literature values and mass spectrum.
[y]Tentatively identified by linear retention index (RI) on HP-5 column, aroma description as compared with standard or literature values.

experienced panelists evaluated odor quality of effluents from the GC column in triplicate and recorded manually. Identification of the aroma-active components was based on the combination of sensory descriptors, Kovats indices (as determined relative to *n*-alkanes from pentane to pentadecane), comparison with known standards, and by GC-MS using the NIST98 library (NIST, Gaithersburg, Md.).

Results and Discussion

The aroma active compounds in the carambola, guava and mango fruits from two different sample preparation techniques are presented in Tables 1, 2, and 3, respectively. Headspace and solvent extraction methods of sample preparation each have their advantages and disadvantages. Headspace volatile concentrations are relatively low and thus difficult to analyze by MS or GC-O when splitting the sample with a GC column, whereas solvent extraction samples can be concentrated by volatilizing the solvent. The problem with the solvent extraction method is the loss of volatiles with boiling points similar to the extraction solvent and compounds being masked by the solvent peak in chromatographic analyses. Therefore, we used both techniques to obtain a more complete aroma profile of these fruit by GC-O. The combined data from two extraction techniques resulted in detection of 53, 48 and 46 aroma active compounds in carambola, guava

and mango, respectively. There was no single character impact compound that contributed to the aroma of these three fruits. Many aroma active compounds in carambola, guava and mango were present at low concentrations that were difficult to detect by MS. Furthermore, these studies also demonstrated that many volatile compounds at various concentration levels had little to no aroma activity.

Carambola. A total of 53 compounds were found to have odor activity in carambola, including four unknowns (Table 1). Results showed that 33 and 37 compounds were identified as being odor-active using SPME and solvent extraction methods, respectively, while 17 compounds were identified by both methods. Methyl benzoate, ethyl benzoate and limonene were the major compounds in carambola (Table 1) in terms of their concentrations, yet only methyl benzoate and ethyl benzoate were aroma active. These ester compounds likely contributed sweet, floral and warm sulfur undertones to the carambola flavor. The aroma active norisoprenoid compounds including β-ionone, β-damascenone, β-damascone, β-ionone, β-ionol, edulan1 and megastigma-4, 6(E), 8(E)-triene in carambola could have contributed sweet honey notes to the flavor.

Guava. For red guava, 48 compounds, including four unknowns, were found to have odor activity (Table 2). There were 40 and 36 compounds identified as being odor-active using SPME and solvent extraction respectively, while 28 compounds were identified by both methods. The compounds in highest concentrations in red guava were hexanal and β-caryophyllene which have aroma activity and contribute to the green fruity and warm floral notes of guava. Sulfur compounds like methanethiol, 2-methyl-3-furanthiol, and mercaptomethylbutyl formate were detected in guava and, no doubt contribute to the unique sulfury note of guava aroma.

Mango. There were 46 compounds including two unknowns in the mango cultivars. For this fruit, 30 and 29 compounds were found using SPME and solvent extraction methods respectively, but only 13 compounds were identified as odor active by both methods (Table 3). This demonstrates that using either method alone would have missed a substantial number of aroma-active compounds. The class of aroma active compounds that contributed a fruity note to mango flavor was of the esters (e.g., ethyl butanoate, ethyl hexanoate) and additionally, many oxygenated volatile compounds were identified as odor-active. Terpene hydrocarbons are reported to be important for mango aroma (Pino et al., 2005), especially the "New World" varieties. However in the present study, esters were the major aroma active compounds followed by aldehydes and alcohols, which contributed to sweet fruity and green notes in this fruit. In fact, the major compound, δ-3-carene, which is considered to be a major aroma-contributing component in mango (MacLeod et al., 1982), and limonene

were found to have no aroma activity in this study; thus they are not listed in the tables.

Conclusion

This study demonstrates the need to use both headspace sampling and solvent extraction to get a good representation of the aroma compounds in a fruit for analysis by GC-O. Fruit volatiles in the highest concentrations were not necessarily the most odor active, and in some cases, were not odor active at all. By far, the esters played the most important role followed by aldehydes, ketones, alcohols, and sulfur-containing compounds. Terpene hydrocarbons were found to have little odor activity with the exception of myrcene, α-terpinene and terpinolene.

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